

# THE EMBRYO SAC OF RICHARDIA AFRICANA KTH.

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(WITH PLATES XXI-XXIII)

Up to the end of last century very little was known of the embryo sacs of the different genera of the Araceae. Since then, however, several American botanists have contributed to our knowledge of the subject, with the result that now we do know something of the development of the embryo sacs. Yet in spite of this work, our knowledge of the family is by no means satisfactory. Perhaps this may be due to the fact that much of the material worked upon has been collected in greenhouses, where conditions are far from normal. The difference in habit between the plants of *Richardia africana* growing in their home in South Africa and those found in greenhouses in England is most striking, and it is possible that conditions which can affect the outward appearance of the plants to such a large extent may be effective in producing abnormalities in the embryo sac.

While this investigation was in progress, Gow (7) published a short account of the embryo sac of *Richardia*. His results differ from mine in so many respects, however, that it seems advisable to publish my results, particularly as my material was obtained from plants in their native habitat.

*Richardia africana* is a native of Cape Colony and St. Helena (5), and flowers freely in damp open places in the Cape Peninsula, where the material for this investigation was collected. The plants are in full flower in August, although young inflorescences may be obtained as early as April.

The chief fixatives used were an alcoholic solution of corrosive sublimate and picric acid, made according to JEFFREY'S formula: Carnoy's fluid and a mixture of three parts of absolute alcohol to one part of glacial acetic acid. A certain amount of shrinking took place in all cases, though in the very young ovaries fixed in the absolute acetic mixture this was practically negligible. Chrom-acetic solutions gave very poor results. The chief stain used

throughout was Haidenhain's iron-haematoxylin, as this gave excellent differentiation in all stages. Good results in the post-fertilization stages were obtained with carthamine (gossypimine) and picric-aniline blue. Many other stains were tried, but none of them was as good as the two already mentioned.

### Ovule and embryo sac

The ovary usually consists of 3 carpels and is trilocular, each loculus containing about 4 not very definitely anatropous ovules arranged in an axile manner. These ovules have the massive basal part of the outer integument so characteristic of the Araceae. The inner integument closes about the time that the megaspore mother cell shows signs of the first division leading to the formation of the embryo sac. The outer integument remains open until endosperm development is well under way.

The two integuments show numerous changes throughout their course of development. The inner one does not attain any great thickness, never being more than 2 or 3 cells thick. Before fertilization, the cells of the innermost layer of this integument, especially those cells in the region round the lower part of the embryo sac, grow rapidly in a plane at right angles to the axis of the ovule (fig. 11*a*). After fertilization, cells of this layer again grow rapidly and gradually lose their flattened form, although they still remain conspicuous by reason of their large size. The chief feature of the outer integument is its great thickness in the chalazal region. It is composed of small uniform cells, with here and there a large cell containing raphides. These large cells are in every way similar to those found in the ovary wall. As the seed matures, intercellular spaces appear in the integument, and the outer surface becomes lobed, particularly at the micropylar end.

The nucellus, as in most other Araceae, is not very permanent tissue, and by the time the egg is ready for fertilization all that remains of it is a cap above the embryo sac and a few cells at the base.

The archesporial cell is differentiated early, before the origin of the second integument. It is easily distinguishable by its denser protoplasm and somewhat larger nucleus. Soon after the appear-

ance of the rudiment of the outer integument, the nucleus of this cell goes into synapsis. Fig. 1 shows the spireme loosening itself from the synaptic knot. The synaptic knot in *Richardia* is a very tight one, and in many ovules in this stage no trace of the spireme could be detected emerging from it. Fig. 2 shows a further stage in the heterotypic division, 12 bivalent chromosomes being arranged on the equatorial plate. The homotypic division follows, thus giving rise to a row of 4 megaspores. Fig. 3 shows stages in the anaphase of this division. In the nuclei represented in this figure it is interesting to note that in the upper nucleus the two halves of one chromosome have not separated, but have remained attached by one end. As a rule sluggish divisions of this type were not observed.

Of the 4 megaspores, only the lowest gives rise to the embryo sac. Fig. 4 shows an ovule in which the uppermost megaspore is beginning to degenerate. In certain cases in which the uninucleate embryo sac is a little larger than the one in this figure, only the remains of 2 megaspores could be detected. It is possible that in these cases only 3 megaspores were formed, although none of my preparations of stages before degeneration shows fewer than 4 megaspores.

Gow (7) states that the spore mother cell develops directly into the embryo sac and that a row of megaspores is not formed. In my material I found that a row of megaspores is invariably formed, and in no case is there anything to lead one to suppose that the embryo sac has originated directly from the megaspore mother cell.

The development of the embryo sac is perfectly normal. In the majority of cases there is a marked polarity. Fig. 5 shows the first division in the embryo sac. In the section represented, only 2 of the degenerating megaspores are to be seen, but a third is present in the next section. Fig. 6 shows the binucleate stage of the embryo sac, and fig. 7 the 4-nucleate stage. Fig. 8 represents the division of these 4 nuclei. It is unfortunate that this was the only case obtained in which the 4 nuclei were dividing, as the polarity so generally met with is absent, and it is not known whether this is the usual state of affairs or not. In subsequent stages shown in my preparations the embryo sac is markedly bipolar.

Fig. 9 shows the upper polar nucleus beginning to move toward the chalazal end of the embryo sac. The next stage is shown in fig. 10, in which the antipodals are showing signs of disintegration. The synergids have not assumed their mature form, but nevertheless may be distinguished from the egg by their position. Early degeneration of the antipodals is usually marked in *Richardia*, although in a few cases they persist, although somewhat degenerate in appearance, up to the time of endosperm formation. In nearly all my preparations showing the mature embryo sac, all that remains of the antipodals is a dark mass of degenerating cells which are hardly distinguishable from the nucellar cells, which at this period often show signs of breaking down. It was not until younger stages were procured that it could be clearly demonstrated that the embryo sac had 8 nuclei, and not 5, as CAMPBELL (4) has described for certain species of *Aglaonema*. However, the stages figured in figs. 8 and 10 prove conclusively that at one period the embryo sac has 8 nuclei.

The stage most commonly met with, and one which evidently persists for some time, is the 5-nucleate one, shown in fig. 11(a, b, c). Here the synergids are quite distinct from the egg, as the cytoplasm of the egg is very much finer. The 2 polar nuclei are imbedded in dense granular protoplasm at the base of the embryo sac, and these nuclei are by far the most conspicuous nuclei in the embryo sac, owing to their larger size and deeper staining capacity. The degenerate antipodals are to be seen at the base of the embryo sac. Figs. 11a and 11b show the halves of 2 of the 3 antipodals, while fig. 11c shows the third.

The 2 polar nuclei fuse some time before fertilization. Fig. 12 shows this fusion taking place in an embryo sac where the 2 nuclei are somewhat smaller than is usually the case.

The position of the polar nuclei at the base of the embryo sac is a constant feature of *Richardia*, and in over 300 slides showing the embryo sac in this stage, none shows the polar nuclei in any position save that at the base of the embryo sac. Gow (7) in fig. 39 of his paper depicts a mature embryo sac which is quite unlike anything that I have come across in my preparations. At the time of maturity the embryo sac has the appearance shown in

fig. 11a. As fertilization has been demonstrated at this stage, it is safe to conclude that the embryo sac is mature. It is possible that Gow's figure was taken from a younger ovule.

### Fertilization

The process of fertilization is rather difficult to demonstrate in *Richardia*. Quite possibly it does not occur in the majority of ovules, as even in its native habitat *Richardia* does not set seed freely. It appears that this failure to set seed is seldom due to a lack of organization of the embryo sac, or to sterility of the pollen grains, but probably may be accounted for by the absence from the plants of an efficient pollinating agent. In this connection it is significant to note that it is rare to find one pollinated ovary alone on an inflorescence. If pollination has occurred, usually most of the ovaries have been pollinated.

CHURCH (5) has reported that small flies and green aphids visit the inflorescence when in cultivation in England, but adds that they do not seem to be of much service in pollination. The same thing, if earwigs be added to the list, occurs in the Cape Peninsula, where the remains of these insects are to be found at the base of inflorescences which obviously have not been pollinated.

A large number of ovaries were collected from inflorescences of widely differing ages. The embryo sac is mature by the time the spathe opens, and persists in this stage for an indefinite length of time. It has even been found in an ovary taken from an inflorescence whose spathe was withering.

Even if fertilization does not occur, the ovaries enlarge. It has not been possible, through lack of opportunity of proving it experimentally, to demonstrate whether the egg is capable of being fertilized the whole time the spathe is open, or whether the fertilization period is restricted to a longer or shorter time dating from the opening of the spathe.

Only one case of undoubted fertilization has been observed. This is shown in fig. 13, in which the pollen tube is seen passing through a synergid and the male nucleus is fusing with the egg nucleus. "Double fertilization" has not been observed. In the lower part of the ovule in which fertilization is being effected, the

primary endosperm nucleus has already divided, and thus there is no means of detecting whether a male nucleus took part in its formation or not. This early stage in endosperm formation is shown in fig. 4, where the antipodals are still distinct.

### Sterile ovules

Gow (7) has reported that in *Richardia* as observed in the Cedar Rapids greenhouses, a large number of ovules are congenitally sterile, producing no embryo sac. In my South African material this does not seem to be the case. In a few ovules no functional embryo sacs were found, but judging from the fact that a group of degenerate cells could be distinguished in the nucellus in each case, it seems probable that an embryo sac had been formed and had collapsed.

### Development of the embryo

Owing to the fact that fertilization had occurred in very few cases, ovules showing young embryos were only occasionally found. Endosperm development precedes that of the embryo, and by the time the small spherical embryo consists of 32 cells, the embryo sac is filled with endosperm.

In one case a 2-celled structure, which had every appearance of being an embryo, had developed at the chalazal end of the embryo sac (fig. 15a). Its protoplasm is fine and dense, making a marked contrast with the coarsely granular protoplasm around the endosperm nuclei which are found in the next section (fig. 15b). The position of the antipodals is indicated by the degenerating cells shown in figs. 15a and 15b. This was the only case in which an embryo was found in any but the normal position, and its origin is not known. In this embryo sac an egg was organized (fig. 15c), although it has evidently not been fertilized.

In all the other ovules the proembryos seen had at least 32 cells and were spherical in shape. Fig. 16 shows a somewhat oblique section passing through an embryo with over 32 cells. The embryo conforms to the type described by both CAMPBELL and GOW for the Araceae.

### Endosperm formation

Endosperm formation proceeds from the base upward. Exactly when cell walls first appear has not been ascertained, but from a comparison of different stages in endosperm development it seems probable that they appear soon after the first 2 or 3 nuclear divisions. Fig. 14, which represents the chalazal end of the embryo sac in which fertilization has been demonstrated, shows the 2 nuclei resulting from the division of the primary endosperm nucleus. Fig. 15*b* shows the division of the lower of these nuclei. Fig. 15 shows the antipodals which have by this time lost all semblance of active cells. Perhaps it is hardly fair to quote this as an instance of the complete disorganization of the antipodals after endosperm formation, as in this embryo sac an abnormal embryo has been formed, but the same thing is seen in fig. 11 (*a, b, c*) and in fig. 17, which represents a slightly later stage in the development of another embryo sac, and in every preparation of embryo sacs made at this stage. It is clear that the antipodals disorganize rapidly, and in the embryo sacs shown in figs. 18 and 19 they are no longer recognizable.

The basal endosperm cells usually have a striking appearance. Three to five of these cells become much larger than any other endosperm cells, and may be seen with the naked eye (figs. 18 and 19). The protoplasm and nuclei of these cells also undergo some change. The protoplasm becomes coarsely granular and has a honeycombed appearance. All nuclear outline is lost, while the nucleolus hypertrophies, shows great vacuolization, and finally fragments, spreading its substance over practically the whole cell. The nuclei of the neighboring endosperm cells often imitate those of the giant cells in behavior. One thing is certain, and that is that these cells are true endosperm cells and have nothing to do with the antipodals.

The function of these cells is not clear. It is interesting to compare them with the antipodal cells described in *Tricyrtis hirta* by IKEDA (10). IKEDA believes that the cytological features exhibited by the antipodals in *Tricyrtis* bear a relation to their nutritive activity. This seems to be true for these endosperm cells of *Richardia*. Everything points to the conclusion that these

cells have a nutritive function. CAMPBELL has suggested that the massive basal part of the outer integument in the Araceae may be physiologically considered as perisperm. As both the inner walls of the outer integument and the embryo sac wall, except at its base, are cutinized, it is obviously impossible for food to pass into the embryo sac except through the enlarged endosperm cells. By the time the embryo is mature these cells have disappeared and much of the food material in the basal part of the integument has been absorbed.

It is a well known fact that cells which are active in nutrition possess nuclei which differ from nuclei in the ordinary resting condition in their larger size, their chromatin aggregations, and appearance of lack of organization. HUIE (8, 9) and ROSENBERG (12) have described this phenomenon in the gland cells in the tentacles of *Drosera*, while MAGNUS (11) has shown that the same thing occurs in the digestive cells of certain orchids having endotrophic mycorrhiza. These facts seem to indicate that a nutritive function is to be ascribed to these basal endosperm cells.

### Discussion

Great variety in development of the embryo sac, even within a single species, has been reported for the Araceae, and it was with a view to finding out in what respects *Richardia africana* resembled the other members of the family that this investigation was undertaken. *Richardia africana*, or *Lantedeschia aethiopica* as ENGLER (6) calls it, belongs to the Philodendroideae, to which group also belong *Homalonema*, *Philodendron*, *Aglaonema*, and *Dieffenbachia*.

According to CAMPBELL and GOW, all these genera, with the exception of certain species of *Algaonema*, show a normal 8-nucleate embryo sac. In a series of papers on the subject, CAMPBELL (1, 2, 4) has dealt with 4 species of *Aglaonema*. He finds that the embryo sac of *A. commutatum* may contain 4-12 nuclei, and shows little uniformity in arrangement. This species has a group of cells at the base of the endosperm bearing some resemblance to that in *Richardia*. In 1900 CAMPBELL suggested that these might originate by division of the antipodals, but later in 1903 inclined to the view that they were of endospermic origin. Gow (7), however, in



1913, without reference to the later paper, confirmed CAMPBELL'S view of 1900.

In 1912 CAMPBELL (4) published the results of his researches on *A. simplex* and *A. modestum*. He believes that there are only 5 nuclei in the embryo sac in these species, this stage arising from the further division of one of the micropylar nuclei in the 4-nucleate stage. The obstacle in the way of accepting CAMPBELL'S view is that he has never been able to demonstrate the supposed nuclear division. My investigations on the embryo sac of *Richardia*, which about the time of maturity has every appearance of being 5-nucleate, have led me to doubt the division of one only of the 4 nuclei.

In many cases the antipodals degenerate almost as soon as formed, and are indistinguishable from the nucellar cells, which also show signs of degeneration. However, it has been possible to show that in *Richardia*, at least, the embryo sac is at one stage 8-nucleate; and in the absence of definite proof to the contrary one cannot but feel that the same may be true for *Aglaonema*. In the fourth species *A. pictum*, CAMPBELL records the presence of cells which look like antipodals, although in the light of his work on *A. modestum* and *A. simplex*, he is inclined to believe that they are of nucellar origin.

In a comparison of *Richardia* with the other genera of the Araceae, mention must be made of *Spathicarpa*, the development of which has been studied by CAMPBELL (2). At the time when the embryo sac is filled with endosperm, it bears a striking resemblance to that of *Richardia*, but in *Spathicarpa* CAMPBELL derives the large cells at the base of the endosperm from the antipodals, which up to the time of fertilization are inconspicuous. In *Richardia* this is not the case, the antipodals being evanescent in character.

### Summary

1. The ovary of *Richardia africana* is usually trilocular and has axile placentation. Four ovules are borne in each loculus.
2. The ovule is not very decidedly anatropous and has two integuments.

3. By the time the embryo sac is mature only a few cells at the apex and base of the nucellus remain.

4. The primary sporogenous cell gives rise directly to a row of 4 megaspores. The embryo sac is derived from the lowest of these.

5. An 8-nucleate embryo sac develops in the normal way.

6. The antipodals usually degenerate early, and when the embryo sac is mature often cannot be distinguished from the nucellus, which also undergoes a certain amount of degeneration.

7. The embryo sac persists for a long time in the stage when only 5 nuclei are distinguishable. The egg apparatus is normal and the 2 large polar nuclei lie in a mass of granular protoplasm at the base of the embryo sac.

8. The proembryo is spherical, with a minute suspensor.

9. In one ovule a 2-celled structure looking like a young embryo was found at the chalazal end of the embryo sac.

10. The endosperm develops from the base upward, and is probably accompanied by wall formation.

11. A few cells at the base of the endosperm are much larger than the rest. They possess hypertrophied nuclei and granular protoplasm. Their function is probably that of passing up food material to the young endosperm and embryo.

In conclusion I am glad to take this opportunity of thanking Dr. PEARSON for suggesting the work and for handing over to me his material and some preparations he had made. I am also indebted to Professor SEWARD for kind permission to carry on this investigation at the Cambridge Botany School, and to Mr. GREGORY for helpful criticism of this paper. I also wish to thank Miss E. L. STEPHENS, who verified certain points for me in connection with the pollination.

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## EXPLANATION OF PLATES XXI-XXIII

### PLATE XXI

- FIG. 1.—Young ovule showing megaspore mother cell with nucleus just coming out of synapsis;  $\times 1210$ .
- FIG. 2.—Formation of equatorial plate in heterotypic division;  $\times 1210$ .
- FIG. 3.—Homotypic division;  $\times 1210$ .
- FIG. 4.—Row of 4 megaspores;  $\times 520$ .
- FIG. 5.—First division of embryo sac nucleus; two degenerating megaspores seen above it;  $\times 520$ .
- FIG. 6.—Two successive sections (*a, b*) showing the 2-nucleate embryo sac;  $\times 520$ .
- FIG. 7.—Four-nucleate embryo sac;  $\times 520$ .
- FIG. 8.—Successive sections (*a, b, c*) showing the division of the nuclei in the 4-nucleate embryo sac;  $\times 520$ .

### PLATE XXII

- FIG. 9.—Upper part of embryo sac showing polar nucleus moving down;  $\times 520$ .
- FIG. 10.—Successive sections (*a, b, c, d*) showing 8-nucleate embryo sac after upper polar nucleus has moved down;  $\times 520$ .
- FIG. 11.—Successive sections (*a, b, c*) showing mature embryo sac with egg (*e*) and 2 synergids (*syn*), the two polar nuclei (*p*), and 3 degenerating antipodals (*ant*);  $\times 520$ .
- FIG. 12.—Fusion of polar nuclei;  $\times 520$ .

FIG. 13.—Fertilization; pollen tube (*pt*) seen in protoplasm of one of the synergids and male nucleus fusing with egg nucleus;  $\times 1210$ .

FIG. 14.—Endosperm after first nuclear division;  $\times 650$ .

PLATE XXIII

FIG. 15*a*.—Young embryo (*emb*) at the chalazal end of the embryo sac; degenerating antipodals (*ant*) below it;  $\times 520$ .

FIG. 15*b*.—Division of the lower of the two endosperm nuclei in the same embryo sac;  $\times 520$ .

FIG. 15*c*.—Unfertilized egg in the same embryo sac;  $\times 520$ .

FIG. 16.—Young proembryo;  $\times 520$ .

FIG. 17.—Two successive sections (*a*, *b*) showing early stage in endosperm development;  $\times 520$ .

FIG. 18.—Endosperm showing enlarged basal cells;  $\times 122$ .

FIG. 19.—Same, in which ordinary endosperm cells have imitated the appearance of the giant cells.